different composition of the volatiles from this fruit compared to those from custard apple precludes valid comparison.

ACKNOWLEDGMENT

The assistance of A. Blofeld, School of Chemistry, Macquarie University, Sydney, in obtaining GC–MS data is gratefully acknowledged.

Registry No. α-Pinene, 80-56-8; camphene, 79-92-5; β-pinene, 127-91-3; myrcene, 123-35-3; α-phellandrene, 99-83-2; α-terpinene, 99-86-5; limonene, 138-86-3; β-phellandrene, 555-10-2; 1,8-cineole, 470-82-6; β-*cis*-ocimene, 3338-55-4; β-*trans*-ocimene, 3779-61-1; *p*-cymene, 99-87-6; ferpinolene, 586-62-9; α-*p*-dimethylstyrene, 1195-32-0; α-cubebene, 17699-14-8; α-copaene, 3856-25-5; linalool, 78-70-6; β-cubebene, 13744-15-5; *trans*-*p*-menth-2-en-1-ol, 35376-40-0; bornyl acetate, 76-49-3; β-elemene, 33880-83-0; terpinen-4-ol, 562-74-3; caryophyllene, 87-44-5; aromadendrene, 72747-25-2; *cis*-menth-2-en-1-ol, 29803-82-5; isopinocarveol, 6712-79-4; humulene, 6753-98-6; α-terpineol, 98-55-5; borneol, 507-70-0; viridiflorene, 21747-46-6; germacrene D, 23986-74-5; bicyclogermacrene, 24703-35-3; δ-cadinene, 483-76-1; globulol, 489-41-8; viridiflorol, 552-02-3; spathulenol, 6750-60-3; T-cadinol, 5937-11-1; T-munrolol, 19912-62-0; δ-cadinol, 36564-42-8; α-cadinol, 481-34-5.

LITERATURE CITED

Engel, K. H.; Tressl, R. *J. Agric. Food Chem.* **1983**, *31*, 796.
Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectral Data Base*; U.S. Government Printing Office: Washington DC, 1980.
Idstein, H.; Herres, W; Schreier, P. *J. Agric. Food Chem.* **1984**, *32*, 383.
Jennings, W. G.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic: New York, 1980.
Likens, S. T.; Nickerson, G. B. *Proc. Am. Soc. Brew. Chem.* **1964**, 5.
MacLeod, A. J.; Pieris, N. M. *J. Agric. Food Chem.* **1981**, *29*, 488.
MacLeod, A. J. Snyder, C. H. *J. Agric. Food Chem.* **1985**, *33*, 380.
Paull, R. E.; Deputy, J.; Chen, N. J. *J. Am. Soc. Hortic. Sci.* **1983**, *108*, 931.
Stenhagen, E.; Abrahamsson, S.; McLafferty, F. W. *Registry of Mass Spectral Data*; Wiley: New York, 1974.
Tressl, R.; Engel, K. H.; Kossa, M.; Kopler, H. *J. Agric. Food Chem.* **1983**, *31*, 892.
Vernin, G.; Metzger, J.; Franisse, D.; Snon, K.; Scharff, C. *Perfum. Flavor.* **1984**, *9*, 71.
Wills, R. B. H.; Poi, A.; Greenfield, H.; Rigney, C. J. *HortScience* **1984**, *19*, 96.

Received for review October 6, 1986. Accepted April 13, 1987.

Binding of Alkanone Flavors to β-Lactoglobulin: Effects of Conformational and Chemical Modification

Timothy E. O'Neill and John E. Kinsella*

Purified β-lactoglobulin B (β-Lg) readily bound the alkanones 2-heptanone, 2-octanone, and 2-nonanone. The β-Lg possessed one primary binding site per monomer, and the binding constants (K) were 150, 480, and 2400 M^{-1} for these compounds, strongly suggesting hydrophobic interactions. Modification of the structure of β-Lg with urea, reduction of disulfide bonds, or ethylation reduced binding of these flavor compounds reflecting the importance of native structure in determining binding affinities.

The interactions of flavors with food components affect the perceived flavors of foods (Solms, 1973; Franzen and Kinsella, 1975; Kinsella, 1981; Damodaran and Kinsella, 1980). The concentration of a flavor in saliva may determine the perceived intensity of food flavors (McNulty and Karel, 1973), and food components that affect the rate or extent of transfer of a flavor component from a food to the saliva influence the perceived flavor.

Proteins can bind flavor compounds and affect the flavor of foods (Gremli, 1974; Beyeler and Solms, 1974; Arai et al., 1970; Franzen and Kinsella, 1974, 1975; Kinsella, 1980; Damodaran and Kinsella, 1981a,b). Some information is available concerning the extent and nature of flavor binding by soy proteins (Damodaran and Kinsella, 1980; Wilson, 1985). Research aimed at understanding the fundamental mechanisms of flavor binding to other proteins is needed. Relationships between the conformational states of proteins, their surface properties, and flavor-binding behavior need to be established before flavor-binding phenomena can be effectively controlled to optimize the impact of added flavors or minimize off-flavors.

β-Lactoglobulin (β-Lg) is a useful model protein for studying flavor protein interactions because its conformation and physical properties are well-defined (McKenzie, 1971; Creamer et al., 1983). In addition, β-Lg possesses measurable binding properties for nonpolar compounds; hence, we selected β-Lg to study its capacity for binding alkanone flavors.

Institute of Food Science, Cornell University, Ithaca, New York 14853.

MATERIALS AND METHODS

Materials. β-Lactoglobulin (β-Lg) was purified from commercial β-lactoglobulin (1× crystallized and lyophilized; Lot 111F-8025, Sigma, St. Louis, MO) by the anion-exchange chromatographic method of Piez et al. (1961). Spectral-grade isooctane was purchased from Fisher Scientific Co. (Fairlawn, NJ). Pure (>98%) 2-nonanone, 2-octanone, and 2-heptanone were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were reagent grade. Distilled, deionized water was used in all experiments.

Protein Solutions. The concentrations of native β-Lg in solution were determined with an absorptivity of 9.55 at 278 nm for a 1% solution (McKenzie, 1971). The concentrations of ethyl-esterified and sulfite-reduced proteins were determined by the method of Lowry et al. (1951) using pure β-Lg as a standard. Protein solutions at concentrations of 1% were made in 20 mM phosphate buffer, pH 6.7, containing sodium azide (0.02%). All protein solutions were passed through a Millipore (Bedford, MA) type AP prefilter before use to remove undissolved materials.

Measurement of Binding. The interaction of alkanones with β-Lg was studied by an equilibrium dialysis method as described by Damodaran and Kinsella

0021-8561/87/1435-0770$01.50/0

(1981a,b). Acrylic cells of equal volume, separated by a membrane (Technilabs) and bolted together, were used. One milliliter of β-Lg solution was placed on one side of the membrane, and 1 mL of buffer containing only a ketone ligand was placed in the other compartment. The cells were then shaken for 20–40 h at 25 °C to attain equilibrium between the flavor and protein. Aliquots were then removed from each compartment, placed in vials with equal volumes of isooctane, and shaken to quantitatively extract the ligand into the isooctane phase. The concentration of the ligand in the isooctane phase was determined by gas chromatography (Damodarn and Kinsella, 1981a,b). The difference in the concentrations of ligand in the respective compartments represented the amount of ligand bound by the protein, and from a molecular mass of 18000 for β-Lg, the number of moles of ligand bound per mole of protein was calculated (Damodaran and Kinesella, 1981a; O'Neill and Kinsella, 1987a,b). The free ligand concentration ([L]) was represented by the ligand concentration on the buffer side of the memberane. Binding data were analyzed by the double-reciprocal plots of Klotz et al. (1946) and Klotz and Urquhart (1948), where $1/\bar{V} = 1/n + 1/nK[L]$, where $\bar{V}$ is the number of moles of ligand bound per mole of protein, [L] is the free ligand concentration, n is the total number of binding sites on the protein molecule, and K is the intrinsic association or binding constant. The free energy of association, ΔG, was calculated from the value of K by $\Delta G = RT \ln K$.

Gas Chromatography. A Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector and Model 7671A automatic sampling device was used. Samples were injected onto a 0.75 mm × 60 m Supelco SP2330 capillary column. Gas flow rates were as follows: hydrogen, 20 mL/min; oxygen, 200 mL/min; carrier nitrogen, 2 mL/min; makeup nitrogen, 40 mL/min. The concentrations were determined from a standard response curve for each alkanone.

Chemical Modification. Purified β-Lg was ethyl esterified by the methods of Mattarella et al. (1983). Protein was suspended on cold ethanol to give a 1% suspension, and concentrated HCl was slowly added to the suspension, while stirring, to give a final HCl concentration of 0.07 M. The mixture was then stirred at 6 °C for approximately 72 h, diluted 1:1 with distilled deionized water, dialyzed exhaustively against 0.001 M HCl, and lyophilized. The isoionic points of the ethyl-esterified and native β-Lg preparations were 8.9 and 5.4, respectively, as determined by the method of Mattaraella et al. (1983).

The two disulfide bonds of β-Lg were reduced by the method of Cole (1967) as modified by Kella and Kinsella (1985). To 0.8 g of purified β-Lg dissolved in 50 mL of water were added 0.5 M ammonium hydroxide, 1.0 mmol of cupric sulfate dissolved in 40 mL of water (pH 9.0), and 10 mL of 0.7 M sodium sulfite. The solution was allowed to stand for 2 h at 25 °C, exhaustively dialyzed against 0.1 M sodium citrate, pH 7.0, to remove copper and against distilled deionized water to remove other salts, and then lyophilized. The complete reduction of disulfide bonds was confirmed by measurement of free sulfhydryl groups using Ellman's reagent as described (Brinegar and Kinsella, 1981).

Fluorescence and UV Difference Spectra. Fluorescence emission spectra of modified and unmodified β-Lg were recorded on a Perkin-Elmer Model 650-40 fluorescence spectrophotometer. Protein solutions (0.020%) were excited at 280 nm, and the fluorescence spectra were scanned from 300 to 400 nm. A slit width of 5 nm was used for both excitation and emission. Ultraviolet difference spectra of sulfite-reduced β-Lg (concentration 2%) were measured in a Cary 219 double-beam spectrophotometer using quartz cells of 1-cm path length.

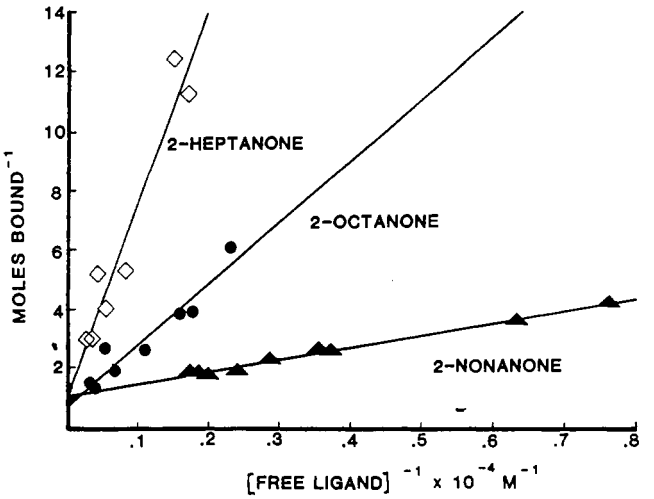

Figure 1. Effect of chain length on the binding of alkanones to β-lactoglobulin B. Binding data are presented in the form of double-reciprocal plots, using a molecular mass of 18000 for β-lactoglobulin B.

Table I. Association Constants and Free Energy of Binding of 2-Alkanones to β-Lactoglobulin B

ligand	K, M^{-1}	ΔG, kcal/mol
2-heptanone	150	−2.98
2-octanone	480	−3.66
2-nonanone	2440	−4.62

Electrophoresis. Native slab polyacrylamide gel electrophoresis (PAGE) (Brinegar and Kinsella, 1981) was used to study protein purity. Gels were run at 24 mA for 3 h at 15 °C, fixed in 10% acetic acid and 50% methanol, stained in 0.10% Coomassie Brilliant Blue R, 30% methanol, 10% acetic acid, and 3% sulfosalicylic acid, and destained in 10% acetic acid and 10% methanol (Brinegar and Kinsella, 1981).

RESULTS AND DISCUSSION

β-Lactoglobulin readily bound the 2-alkanones tested. Double-reciprocal plots for binding of 2-heptanone, 2-octanone, and 2-nonanone (Figure 1) revealed similar values for the y intercept for all three compounds, indicating one primary binding site for alkanones. The slopes of these lines correspond to the value of $1/nK$ from which the binding constants and corresponding free energies of association were obtained (Table I). These data indicate that the binding affinity of aliphatic ketones for β-Lg increased with chain length of the ligand. The free enery for the association of 2-alkanones with β-Lg changed by about −700 cal with an increase in chain length from 2-heptanone to 2-octanone and by about −1000 cal from 2-octanone to 2-nonanone. The effect of chain length upon the free energy of binding suggests that the association is primarily hydrophobic in nature as reported for bovine serum albumin and soy proteins (Damodaran and Kinsella, 1980, 1981a,b; O'Neill and Kinsella, 1987). The free energy changes, for an increase in chain length of one methylene group were −550 and −600 cal, respectively, for the binding of these alkanones to bovine serum albumin (Damodaran and Kinsella, 1980) and to soy protein (Damodaran and Kinsella, 1981a,b). Robillard and Wishnia (1972) reported that the increase in free energy of association was 1.07 kcal/methylene residue for the binding of alkanes to β-LgA.

Table II. Parameters for the Binding of Nonpolar Compounds to β-Lactoglobulin

compound	n (per dimer)	K, M^{-1}	reference
retinol	2	5×10^7	Fugate and Song (1980)
stearate	2	1.7×10^5	Spector and Fletcher (1969)
palmitate	2	6.8×10^5	Spector and Fletcher (1969)
laurate	2	0.5×10^5	Spector and Fletcher (1969)
oleate	2	0.4×10^5	Spector and Fletcher (1969)
heptane	2	0.48×10^6	Mohammadzadeh et al. (1969)
iodobutane	2	2.8×10^3	Wishnia and Pinder (1966)
pentane	2	7.1×10^3, 6.2×10^2	Wishnia and Pinder (1966)
butane	2	1.7×10^3, 5.8×10^2	Wishnia and Pinder (1966)
2-heptanone	2	0.15×10^3	present study
2-octanone	2	0.50×10^3	present study
2-nonanone	2	2.44×10^3	present study
N-methyl-2,6 ANS	2	3.4×10^5, 2.0×10^4	Lovrien and Anderson (1969)
n-octyl benzene-p-sulfonate	2–3	6.3×10^4	Hill Briggs (1956)
SDS	3	3.1×10^5	Ray and Chatterjee (1967)
methyl orange	2	0.20×10^4 (pH 5.4)	Ray and Chatterjee (1967)

The binding constants for the interaction of aliphatic ketones with β-Lg are higher than those for bovine serum albumin or soy protein. A binding constant of 570 M^{-1} and five to six binding sites per molecule of protein was observed for the interaction of 2-nonanone with soy protein, assuming a molecular weight of 100 000 (Damodaran and Kinsella, 1981). On the same molecular weight basis, β-Lg would have approximately five binding sites and a binding affinity of 2440 M^{-1}, approximately 4 times that for soy protein (O'Neill and Kinsella, 1987). Conceivably β-Lg, by virtue of its strong binding affinity (and depending on its concentration), could affect the perceived flavors, of foods.

The tertiary structure of β-Lg reveals a hydrophobic core, which is accessible from the exterior of the protein molecule (Sawyer et al., 1985). This sheltered hydrophobic pocket apparently is the primary binding site on the β-Lg molecule for a variety of nonpolar molecules (Table II), e.g. alkanes (Wishnia and Pinder, 1966), fatty acid (Spector and Fletcher, 1969), sodium dodecyl sulfate (McMeekin et al., 1949; Seibles, 1969; Ray and Chatterjee, 1967), *N*-methyl-2-anilino-6-naphthalenesulfonic acid (Lovrien and Anderson, 1969), and retinol (Fugate and Song, 1980). Robillard and Wishnia (1972) demonstrated, through competitive binding studies that the binding of alkanes and SDS to β-Lg occurs at the same hydrophobic site. It is assumed that 2-alkanones also bind to this single hydrophobic binding site.

Effect of Urea. Hydrophobic binding of ligands by proteins in dependent upon the existence of discrete hydrophobic regions within the protein molecule, which must be accessible to the ligand. Protein denaturants, such as urea, cause unfolding of the tertiary structure of proteins, therby altering the structure of hydrophobic regions and hydrophobic binding. In the presence of urea (which had a negligible effect on ligand solubility) the slopes of the double-reciprocal binding plots increased with urea concentration corresponding to a decrease in binding affinity of β-Lg for 2-nonanone (Figure 2). However, the number of binding sites remained constant over the range of urea concentrations used. The results suggest that urea causes a partial unfolding of β-Lg molecule, which does not destroy the primary binding site but reduces the hydrophobic binding region, resulting in reduced binding affinity.

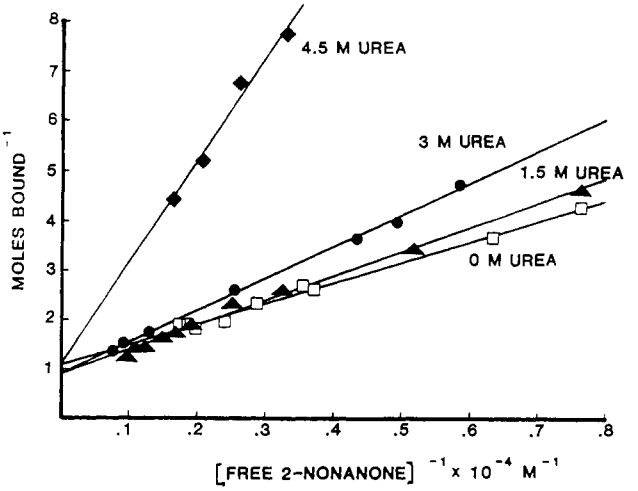

Figure 2. Effect of urea on the binding of 2-nonanone to β-lactoglobulin B.

X-ray crystallographic data indicate that tryptophan is located in the interior of the β-Lg molecule in the proximity of the site for the binding of nonpolar molecules (Sawyer et al., 1985). Fugate and Song (1980) determined that a tryptophan residue is involved in the binding of retinol to β-Lg. Therefore, fluorescence spectra of β-Lg were monitored to ascertain whether a relationship existed between conformation of β-Lg and binding of alkanones.

Native β-Lg exhibited maximum fluorescence intensity at 335 nm, which is consistent with tryptophan being the primary source of fluorescence emission (Figure 3). There was little change in wavelength of maximum fluorescence intensity of β-Lg up to 3 M urea. However, the maximum fluorescence intensity increased with a red shift in maximum fluorescence emission in 4–8 M urea. This is consistent with a shift in wavelength when tryptophan residues become exposed to the aqueous environment from the relatively sheltered anhydrous apolar interior of a protein molecule (Stryer, 1968). The increase in fluorescence intensity is consistent with observation that the fluorescence intensity of tryptophan is enhanced (up to 20%) upon exposure to urea (Kronman and Holmes, 1971). These authors also reported that fluorescence of β-Lg is enhanced in the presence of 6 M guanidine hydrochloride, which does not enhance the fluorescence of free tryptophan. This suggests that urea increases fluorescence intensity by producing changes in the interactions of tryptophan with the side chains of other amino acids and/or by inducing an increased exposure of tryptophan side chains to the solvent.

The relationship between the ligand binding affinity and urea-induced conformational changes (Figure 4) indicates that though there was little change in maximum wavelength of fluorescence emission in 0–3 M urea, the binding constant progressively decreased with the concentration of urea. At urea concentrations of 4 M and above, where a red shift in the maximum wavelength of emission became more pronounced, there was a concomitant larger decrease in binding affinity, e.g. at 4.5 M urea. Urea may increase the solubility of hydrocarbons in water through the alteration of water structure (Wetlaufer et al., 1964) and

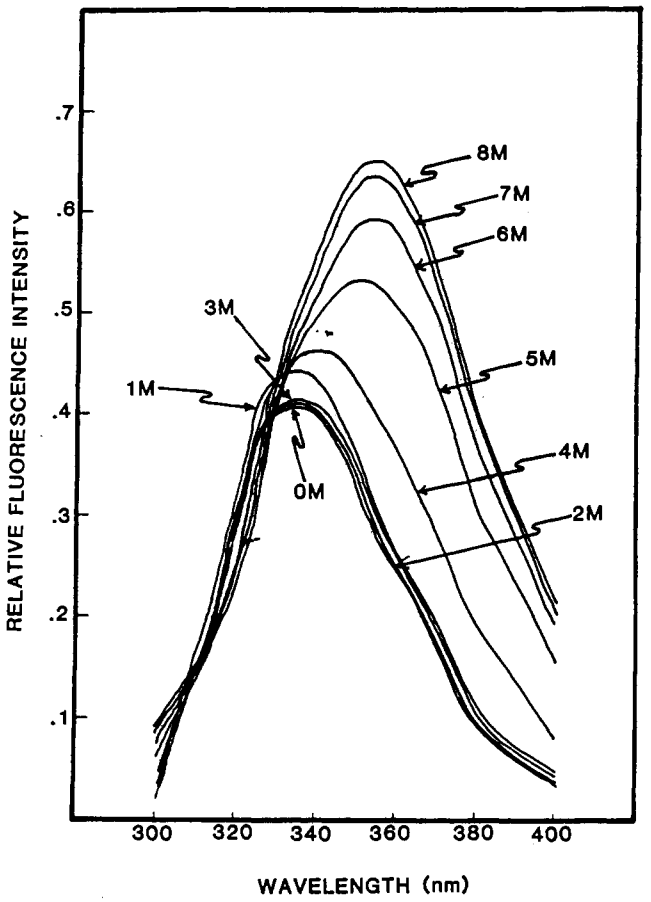

Figure 3. Effects of increasing urea concentrations on the fluorescence spectra of β-lactoglobulin B in 20 mM phosphate buffer, pH 6.7.

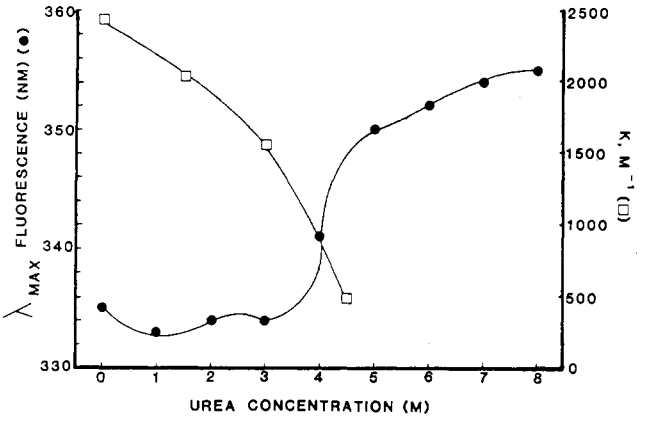

Figure 4. Relationship between wavelength of maximum fluorescence (emission maximum) and binding affinity of β-lactoglobulin B for 2-nonanone.

thereby reduce the driving force for the hydrophobic interaction of 2-nonanone with β-Lg and reduce binding. To determine whether this occurred, the solubility of 2-nonanone in urea solutions of varying concentrations and the partitioning of 2-nonanone from these solutions into isooctane were determined. The solubility of 2-nonanone in the urea solutions was slightly increased, but the partitioning of 2-nonanone between isooctane and the aqueous phase was not significantly affected (data not shown). This indicates that the reduction of binding by β-Lg is caused primarily by the effects of urea on the conformation of the protein, which results in a change in the hydrophobic binding site.

In constrast to alkanones, the binding of retinol to β-Lg is apparently unaffected by the presence of 8 M urea

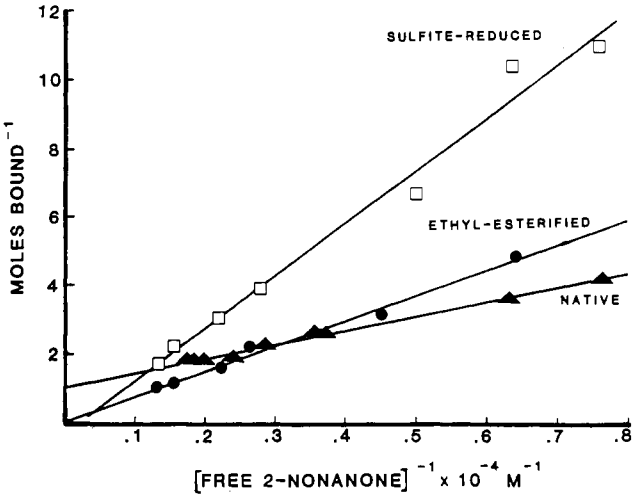

Figure 5. Alteration of binding characteristics of β-lactoglobulin for 2-nonanone following ethyl-esterification or disulfite reduction.

(Fugate and Song, 1980), despite the fact that this concentration of urea may be sufficient to produce significant denaturation of the native β-Lg structure (Schellman, 1958; Kauzman and Simpson, 1953; Pace and Tanford, 1968). This suggests that the binding of retinol to β-Lg may involve a mechanism different from the binding of 2-nonanone, reflecting perhaps an interaction between the β-ionone ring of retinol and aromatic amino acids in the binding site.

Effect of Chemical Modification. Chemical modification of β-LgB by ethyl-esterification and reduction of disulfide bonds with sodium sulfite resulted in dramatically different binding behavior (Figure 5). The apparent binding of 2-nonanone was markedly lower than with the native protein. In addition, the intercepts of the double-reciprocal plots for the modified proteins reflect a large number of binding sites for 2-nonanone following modification. In order to elucidate the manner in which these modifications affected the nature of 2-nonanone binding, some spectroscopic properties of the modified proteins were determined.

The optical density of native, esterified, and sulfite-reduced β-Lg solutions (1%) at 600 nm were 0.007, 0.454, and 0.010, respectively, reflected high turbidity in the ethyl-esterified protein solution, but little in the case of the native or sulfite-reduced protein solutions. The turbidity indicated aggregation of the ethyl-esterified β-Lg (Mattarella et al., 1983). Hence, the ethyl-esterification of carboxyl groups in β-Lg destabilizes the native conformation, causing substantial unfolding and aggregation (Richardson, 1985). These aggregates when dispersed in solution exhibited different binding behavior. Conceivably the presence of aggregates introduced a heterogeneity of binding species into the system, and both physical adsorption and entrapment, in addition specific binding to hydrophobic sites, may have occurred.

The fluorescence spectra of native and sulfite-reduced β-Lg showed that the wavelength of maximum fluorescence emission was shifted from 335 nm for the native protein to 343 nm for the sulfite-reduced protein with a concomitant increase in maximum fluorescence intensity (data not shown). This indicates that reduction of the disulfide bonds caused a change in protein conformation, affecting the environment of the tryptophan residues. This was confirmed by the UV difference spectra for the reduced protein, which showed that absorption was reduced in the 260–310-nm region indicating a change in the environment of the chromophoric aromatic amino acid residues. Thus,

sulfite modification induces a significant unfolding of the native structure that altered 2-nonanone binding.

These data demonstrate that the binding of flavor compounds to β-Lg is affected by the conformational states of proteins as reported earlier for bovine serum albumin (Kinsella, 1980) and soy proteins (Damodaran and Kinsella, 1981a,b). Chemical or physical changes that alter the conformational states produce marked changes in the flavor-binding characteristics of proteins. Future research should be directed toward obtaining a better understanding of the relationship between protein structure and the binding of flavor molecules. In this regard β-Lg is a good model for food protein because its structure and conformation are very well described (Sawyer et al., 1985; Creamer, 1983; McKenzie, 1971). In addition β-Lg has good binding properties as summarized in Table II. Noteworthy from the data (Table II) is its extremely high affinity for retinol, which reflects a considerable homology between the structures of β-Lg and retinol binding protein (Perviaz and Brew, 1985) and may reflect a physiological function of β-Lg as a carrier or retinol (Perviaz and Brew, 1985).

ACKNOWLEDGMENT

Supported in part by Dairy Research Foundation, Wisconsin Milk Marketing Board, and a special award from General Foods to J.E.K.

Registry No. 2-Heptanone, 110-43-0; 2-octanone, 111-13-7; 2-nonanone, 821-55-6.

LITERATURE CITED

Arai, S.; Noguchi, M.; Yamashita, M.; Kato, H.; Fujimaki, M. *Agric. Biol. Chem.* **1970**, *34*, 1569.
Beyeler, M.; Solms, J. *Lebensm.-Wiss. Technol.* **1974**, *7*, 217.
Brinegar, A. C.; Kinsella, J. E. *Int. J. Peptide Protein Res.* **1981**, *18*, 18.
Cole, R. D *Methods Enzymol.* **1967**, *11*, 206.
Creamer, L. *Arch. Biochem. Biophys.* **1983**, *227*, 98.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1980**, *28*, 567.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1981a**, *29*, 1248.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1981b**, *29*, 1253.
Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.
Franzen, K. L.; Kinsella, J. E. *J. Agric. Food Chem.* **1974**, *22*, 675.
Franzen, K. L.; Kinsella, J. E. *Chem. Ind. (London)* **1975**, *21*, 505.
Fugate, R. D.; Song, P. S. *Biochem. Biophys. Acta* **1980**, *625*, 28.
Gremli, H. A. *J. Am. Oil Chem. Soc.* **1974**, *51*, 95A
Hill, R. M.; Briggs, D. R. *J. Am. Chem. Soc.* **1956**, *78*, 1590.
Kauzman, W.; Simpson, R. B. *J. Am. Chem. Soc.* **1953**, *75*, 5154.
Kella, N.; Kinsella, J. E. *Int. J. Peptide Protein Res.* **1985**, *11*, 251.
Kinsella, J. E. In *The Analysis and Control of Less Desirable Flavors in Foods and Beverages*; Charalambous, G., Ed.; Academic: New York 1980.
Klotz, I. M.; Urquhart, J. M. *J. Am. Chem. Soc.* **1948**, *71*, 1597.
Klotz, I. M.; Walker, F.; Pivan, R. B. *J. Am. Chem. Soc.* **1946**, *68*, 1486.
Kronman, M. J.; Holmes, L. G. *Photochem. Photobiol.* **1971**, *14*, 113.
Lovrien, R.; Anderson, W. *Arch. Biochem. Biophys.* **1969**, *131*, 139.
Lowry, O. H.; Rosebrough, N. .; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Mattarella, N. L.; Creamer, L. K.; Richardson, T. *J. Agric. Food Chem.* **1983**, *31*, 968.
McKenzie, H. A. In *Milk Proteins. Chemistry and Technology*; McKenzie, H. A., Ed.; Academic: New York, 1971; Vol. II.
McNulty, P.; Karel, M. *J. Food Technol.* **1973**, *8*, 309–318.
McMeekin, T. L.; Polis, B. D.; DellaMonica, E. S.; Custer, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 3606.
Mohammadzadeh, K.; Feeney, R. E.; Smith, L. M. *Biochem. Biophys. Acta* **1969**, *194*, 246.
O'Neill, T.; Kinsella, J. E. *J. Food Sci.* **1987**, *53*, 98.
Pace, N. C.; Tanford, C. *Biochemistry* **1968**, *7*, 198.
Piez, K.; Davie, E. W.; Folk, J. E.; Gladner I. A. *J. Biol. Chem.* **1961**, *236*, 2912.
Perviaz, S.; Brew, K. *Science (Washington, D.C.)* **1985**, *228*, 335.
Ray, A. P. Chatterjee, R. In *Conformations of Biopolymers*; Ramachandran, G. N., Ed.; Academic: New York, 1967.
Richardson, T. *J. Dairy Sci.* **1985**, *68*, 2753.
Robillard, K. A.; Wishnia, A. *Biochemistry* **1972**, *11*, 3835.
Sawyer, L.; Papiz, M. .; North, A. C. T. *Biochem. Soc. Trans.* **1985**, *30*, 395.
Seibles, T. S. *Biochemistry* **1969**, *8*, 2949.
Schellman, J. A. *C. R. Seances Acad. Sci., Ser. C.* **1958**, *30*, 395.
Solms, J. *Can. Inst. Food Sci. Technol. J.* **1973**, *6*, A10.
Spector, A. A.; Fletcher, J. E. *Lipids* **1969**, *5*, 403.
Stryer, L. *Science (Washington, D.C.)* **1968**, *162*, 526.
Wetlaufer, D. B.; Malik, S. K.; Stoller, L.; Coffin, R. L. *J. Am. Chem. Soc.* **1964**, *865*, 508.
Wilson, L. In *Proceedings of the World Soybean Conference III*; Shibles, R., Ed.; Westview: Boulder, CO, 1985; p 158.
Wishnia, A.; Pinder, T. W. *Biochemistry* **1966**, *5*, 1534.

Received for review April 21, 1986. Revised manuscript received September 19, 1986. Accepted May 18, 1987.

Principal Constituents of Black Truffle (*Tuber melanosporum*) Aroma

Thierry Talou,* Michel Delmas, and Antoine Gaset

A dynamic headspace technique was employed for isolating black perigord truffle (*Tuber melanosporum*) aroma volatiles using a Tenax trap. The volatiles eluted from the trap by heat desorption were analyzed by capillary gas chromatography and coupled capillary gas chromatography–mass spectrometry. A total of 14 volatiles was identified, from which 9 compounds could be described for the first time as black perigord truffle aroma constituents. Quantitatively, alcohols were predominant.

Truffles are subterranean edible fungi (order Tuberales) that grow in various parts of Europe, particularly in France. The black perigord truffle (*Tuber melanosporum*) is a blackish fruit with finely veined flesh.

Its flavor is particularly praised by mushroom fanciers. The aroma impression has been described as slightly sulfurous (Andreotti and Casoli, 1968), but little has been published about black perigord truffle volatiles.

Recent studies were carried out by Ney and Freitag

Laboratoire de Chimie des Agroressources, Ecole Nationale Superieure de Chimie, Institut National Polytechnique de Toulouse, 31077 Toulouse Cedex, France.

0021-8561/87/1435-0774$01.50/0